

WHAT IS CLAIMED IS:

1. A method of generating ascorbic acid, comprising:
culturing a *Kluyveromyces* spp. or a *Zygosaccharomyces* spp. yeast in a medium
comprising an ascorbic acid precursor, thereby forming ascorbic acid, and
5 isolating the ascorbic acid.
2. The method of claim 1, wherein the yeast is a *Z. bailii* or a *K. lactis*.
3. The method of claim 2, wherein the yeast is *Z. bailii* strain ATCC 60483 or
10 *K. lactis* strain PM6-7A.
4. The method of claim 1, wherein the ascorbic acid precursor is L-galactose; D-glucose; L-galactono-1,4-lactone; or L-gulonono-1,4-lactone.
- 15 5. The method of claim 1, wherein the isolating step comprises lysing the yeast.
6. The method of claim 5, wherein the isolating step further comprises centrifugation, filtration, microfiltration, ultrafiltration, nanofiltration, liquid-liquid extraction, crystallization, enzymatic treatment with nuclease or protease, or
20 chromatography.
7. A method of generating ascorbic acid, comprising:
 - a) obtaining a recombinant yeast capable of converting an ascorbic acid precursor into ascorbic acid
 - 25 b) culturing the recombinant yeast in a medium comprising an ascorbic acid precursor, thereby forming ascorbic acid, and
 - c) isolating the ascorbic acid.
8. The method of claim 7, wherein the yeast belongs to the genus *Saccharomyces*,
30 *Zygosaccharomyces*, *Candida*, *Hansenula*, *Kluyveromyces*, *Debaromyces*, *Nadsonia*, *Lipomyces*, *Torulopsis*, *Kloeckera*, *Pichia*, *Schizosaccharomyces*, *Trigonopsis*,

Brettanomyces, *Cryptococcus*, *Trichosporon*, *Aureobasidium*, *Lipomyces*, *Phaffia*, *Rhodotorula*, *Yarrowia*, or *Schwanniomyces*.

9. The method of claim 8, wherein the yeast belongs to the species *S. cerevisiae*,
5 *K. lactis*, or *Z. bailii*.

10. The method of claim 9, wherein the yeast is selected from *S. cerevisiae* strain GRF18U; *S. cerevisiae* strain W3031B; *K. lactis* strain PM6-7A; or *Z. bailii* strain ATCC 60483.

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11. The method of claim 7, wherein the yeast is functionally transformed with a coding region encoding a first enzyme selected from L-galactose dehydrogenase (LGDH), L-galactono-1,4-lactone dehydrogenase (AGD), D-arabinose dehydrogenase (ARA), D-arabinono-1,4-lactone oxidase (ALO), or L-gulonono-1,4-lactone oxidase
15 (RGLO).

12. The method of claim 11, wherein the LGDH enzyme has at least about 70% similarity with SEQ ID NO:11; the AGD enzyme has at least about 70% similarity with SEQ ID NO:1 or SEQ ID NO:3; the ARA enzyme has at least about 70% similarity with
20 SEQ ID NO:20; the ALO enzyme has at least about 70% similarity with SEQ ID NO:5 or SEQ ID NO:7; or the RGLO enzyme has at least about 70% similarity with SEQ ID NO:9.

13. The method of claim 11, wherein the LGDH enzyme has at least about 70%
25 identity with SEQ ID NO:11; the AGD enzyme has at least about 70% identity with SEQ ID NO:1 or SEQ ID NO:3; the ARA enzyme has at least about 70% identity with SEQ ID NO:20; the ALO enzyme has at least about 70% identity with SEQ ID NO:5 or SEQ ID NO:7; or the RGLO enzyme has at least about 70% identity with SEQ ID NO:9.

30 14. The method of claim 11, wherein the coding region encoding the LGDH enzyme has at least about 70% identity with SEQ ID NO:12; the coding region encoding the

AGD enzyme has at least about 70% identity with SEQ ID NO:2 or SEQ ID NO:4; the coding region encoding the ARA enzyme has at least about 70% identity with SEQ ID NO:21; the coding region encoding the ALO enzyme has at least about 70% identity with SEQ ID NO:6 or SEQ ID NO:8; or the coding region encoding the RGLO enzyme has at least about 70% identity with SEQ ID NO:10.

15. The method of claim 11, wherein the yeast is functionally transformed with a coding region encoding ALO.

10 16. The method of claim 11, wherein the yeast is functionally transformed with a coding region encoding ARA.

17. The method of claim 16, wherein the ARA comprises the amino acid sequences GXXXXXAXXXXXXEXXXG (SEQ ID NO:13) and GXXN (SEQ ID NO:26).

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18. The method of claim 11, wherein the coding region is linked to a promoter active in the yeast.

19. The method of claim 18, wherein the promoter is the *S. cerevisiae* triosephosphate isomerase (TPI) promoter.

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20. The method of claim 11, wherein the coding region was isolated from *A. thaliana*, *S. cerevisiae*, or *R. norvegicus*.

25 21. The method of claim 20, wherein the coding region encoding LGDH was isolated from *A. thaliana*, the coding region encoding ALO was isolated from *S. cerevisiae*, the coding region encoding AGD was isolated from *A. thaliana*, the coding region encoding ARA was isolated from *S. cerevisiae*, or the coding region encoding RGLO was isolated from *R. norvegicus*.

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22. The method of claim 11, wherein the AGD enzyme comprises a signaling peptide.

23. The method of claim 11, wherein the AGD enzyme does not comprise a signaling peptide.
- 5 24. The method of claim 11, wherein the yeast is functionally transformed with a coding region encoding a second enzyme other than the first enzyme, wherein the second enzyme is selected from LGDH, AGD, ARA, ALO, or RGLO.
- 10 25. The method of claim 24, wherein the coding region encoding the second enzyme is linked to a promoter active in the yeast.
26. The method of claim 25, wherein the promoter is the *S. cerevisiae* triosephosphateisomerase (TPI) promoter.
- 15 27. The method of claim 11, wherein the recombinant yeast further comprises at least one coding region encoding an enzyme associated with the conversion of a carbon source to L-galactose.
- 20 28. The method of claim 7, wherein the ascorbic acid precursor is selected from L-galactono-1,4-lactone; D-glucose; L-gulono-1,4-lactone; or L-galactose.
29. The method of claim 7, wherein the isolating step comprises lysing the yeast.
- 25 30. The method of claim 29, wherein the isolating step further comprises centrifugation, filtration, microfiltration, ultrafiltration, nanofiltration, liquid-liquid extraction, crystallization, enzymatic treatment with nuclease or protease, or chromatography.
- 30 31. The method of claim 7, wherein the recombinant yeast accumulates L-ascorbic acid in the medium at levels greater than background.

32. The method of claim 31, wherein the isolating step comprises chromatography, activated carbon, microfiltration, ultrafiltration, nanofiltration, liquid-liquid extraction, or crystallization.
- 5 33. The method of claim 7, wherein the recombinant yeast produces ascorbic acid with a yield greater than about 35% from a precursor.
34. A method of stabilizing ascorbic acid in a medium, comprising:
culturing a yeast in the medium.
- 10 35. A *S. cerevisiae*, wherein the *S. cerevisiae* is functionally transformed with a coding region encoding D-arabinose dehydrogenase (ARA).
36. A *S. cerevisiae*, wherein the *S. cerevisiae* is functionally transformed with a
15 coding region encoding L-galactono-1,4-lactone dehydrogenase (AGD).
37. A *S. cerevisiae*, wherein the *S. cerevisiae* is functionally transformed with a coding region encoding D-arabinono-1,4-lactone oxidase (ALO).
- 20 38. A *S. cerevisiae*, wherein the *S. cerevisiae* is functionally transformed with a coding region encoding L-galactono-1,4-lactone dehydrogenase (AGD) and a coding region encoding L-galactose dehydrogenase (LGDH).
39. A *S. cerevisiae*, wherein the *S. cerevisiae* is functionally transformed with a
25 coding region encoding D-arabinono-1,4-lactone oxidase (ALO) and a coding region encoding L-galactose dehydrogenase (LGDH).
40. A *S. cerevisiae*, wherein the *S. cerevisiae* is functionally transformed with a coding region encoding D-arabinono-1,4-lactone oxidase (ALO) and a coding region
30 encoding D-arabinose dehydrogenase (ARA).